A special medium extends the viability of organisms in swab specimens during prolonged transport time from the patient to Alberta's central public health laboratory, which serves an area of about 200,000 square miles.

Transport Medium for Specimens in Public Health Bacteriology

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DELAY during the transmission of specimens from source to laboratory most noticeably affects delicate organisms which survive poorly over a period of 24 to 48 hours. Such organisms are *Neisseria gonorrhoeae*, *Haemophilus pertussis*, and *Trichomonas vaginalis*, and also *Shigella* species when specimens are obtained on rectal swabs. The pyogenic cocci common in throat inflammation and superficial infection are usually more resistant but may be significantly affected if few in number or if transport time is prolonged. Such conditions can readily arise during epidemiological investigation carried out in remote areas.

The problem has been variously tackled in the past. Two major devices have been employed. First, cooling or freezing, which slows normal bacterial metabolism, has been used to extend viability and at the same time to diminish bacterial competition. Second, transport on culture media has been employed to increase viability by encouraging multiplication, but this necessitates selective agents to limit bacterial competition. Both these methods have significant disadvantages. Methods of chilling or freezing are troublesome in initial application and add considerable additional weight and cost during transport. Methods of transport in selective culture media demand a medium appropriate to each bacterial pathogen and are limited by the availability of specific selective agents which will work equally well under a variety of environmental influences and over a greatly variable time period. Such agents do not exist for most bacteria.

A transport medium must be simple in clinical use, easy in laboratory manipulation, cheap, and of wide applicability.

The transport medium to be described has all these virtues. Introduced by Stuart (*1*) primarily for the transport of swabs for gonococcal culture, it was applied by Moffett, Young, and Stuart (*2*) to the investigation of similar material from clinics mostly within 24-hour transport distance, and later by Stuart, Toshach, and Patsula (*3*) to the greater transport distances found in Alberta. Its value for this purpose has been confirmed by L. LeMinor, S. LeMinor, and Combes (*4*) from the Pasteur Institute, Paris, France; by Wilkinson (*5*), from the Whitechapel Clinic, London, England; by Alin (*6*), from the State Public Health Laboratory, Sweden; and by DeBoynton (*7*), Los Angeles County Health Depart-

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ment, Calif., but irregularities in performance have been detected by Cradock-Watson, Shooter, and Nicol (*8*).

Its coincidental value for the preservation of living trichomonads in similar material was emphasized by several of the above workers. Alexander (*9*), in Liverpool, England, emphasized its general value for gynecological specimens. The applicability of this transport medium to other bacteria was indicated first by Stuart (*10*), who dealt particularly with meningococci and *Shigella* species and gave some preliminary information on the satisfactory transmission of *H. pertussis* and other respiratory pathogens. Cooper (*11*), in Australia, produced ample evidence to indicate the value of this transport method for all respiratory and other pathogens examined, while Stokes (*12*) emphasized its value in the transmission of anaerobic bacteria to a central reference laboratory. Many public health laboratories across Canada now use the method routinely for all bacterial infections when specimens have to be transported over considerable distances.

Rationale of Method

The transport method is based on two main premises explained more extensively in earlier work. Briefly these are, first, that oxidation is an important cause of bacterial death during transport and, second, that elimination of all nutrients from a transport medium is the simplest way of maintaining the bacterial status quo in specimens. Thus bacteria, if kept alive by the elimination of oxygen, remain in the same relative proportions in which they existed in the original material from the patient. The applicability of these premises to all bacteria also obviates the necessity for using different transport culture media to suit different bacteria and makes unnecessary the frequently hopeless search for suitably specific bacteriostatic agents to control unwanted bacterial multiplication during transport. Simplicity of clinical application insures that the method is highly acceptable to physicians.

The transport medium is a soft water-agar gel, hard enough to prevent agitation during transport but soft enough to avoid cracking when a swab is pushed into it. Sodium thioglycolate was selected experimentally as the best reducing agent, but is used as thioglycolic acid, chiefly because measuring is simpler than weighing. Conversion to the sodium salt is achieved by adding NaOH to the medium, the pH being stabilized by a phosphate buffer. Calcium, as calcium chloride, was found decidedly beneficial, but its presence necessitated the use of glycerophosphate as a buffer. Calcium glycerophosphate is soluble to about 2 percent, whereas calcium phosphate is almost completely insoluble. A reduction indicator is considered desirable and methylene blue was found decidedly better than resazurin. Acid production by swab sticks and swabs is of occasional detrimental significance, but is eliminated by boiling them in buffer solution before issue. Of major importance, however, is the impregnation of these swabs with charcoal, the rationale of which has been fully described (*3*). This charcoal impregnation neutralizes an incompletely identified inhibitor of bacterial viability, regularly present in variable

Table 1. Results of parallel smear and culture examinations for gonorrhea in females

Year	Number of specimens	Number of positives				Percent positive	
		Total positive	Culture positive, smear positive	Culture positive, smear negative	Culture negative, smear positive	By culture	By smear
1952	2, 546	323	144	143	36	89	56
1953	2, 945	331	130	151	50	85	54
1954	3, 039	411	180	182	49	88	56
1955	3, 243	492	204	238	50	90	52
1956	3, 513	482	180	246	56	88	49
1957	5, 042	705	204	452	49	93	36

Transport Method

Transport Medium

Dissolve 6 gm. Bacto agar in 1,000 ml. chlorine-free distilled water. At the same time to another 900 ml. distilled water, add 2 ml. thioglycolic acid (Difco), 12–15 ml. $\frac{N}{1}$ NaOH (to bring to approximately pH 7.2; the amount is selected according to local experience to avoid as far as possible any final pH adjustment), 100 ml. solution of sodium glycerophosphate (commercial, 20 percent w/v in water), and 20 ml. solution of $CaCl_2$ (1 percent w/v in water). Add this mixture to the original melted agar. Check pH and if necessary adjust to pH 7.3–7.4. Add 4 ml. methylene blue (0.1 percent in water). Mix well and dispense in ¼-oz. screw-capped bottles, filling to capacity. Screw caps on securely, but not tightly. Sterilize in flowing steam, avoiding overcrowding, for 1 hour. Tighten caps immediately following sterilization. After cooling, the transport medium should be colorless.

NOTE: Distillate from chlorinated water occasionally contains significant amounts of free chlorine. This must be checked rigorously. We pass all distilled water through an ion-exchange resin column before use. This water is used for all the above preparation work.

Swabs

Prepare neat swabs with good quality absorbent cotton and applicator sticks. Prepared swabs are boiled in Sorensen's phosphate buffer solution $\left(\frac{M}{15}\right)$ pH 7.4 (approximately 500 swabs standing in 100 ml. buffer in 1,000 ml. beaker, boiled 5 minutes). Remove, shake off excess moisture, then dip in a 1 percent suspension of finely powdered charcoal in water. We use activated charcoal (B.D.H.), but animal charcoal (Cenco), blood charcoal (B.D.H.) and Norit have been used successfully when powdered sufficiently fine. Swirl swabs around to coat thoroughly with charcoal (swabs should be quite black when wet). Shake off excess moisture, place in cotton-plugged tubes, dry in oven, and sterilize.

Instructions Sent With Outfits

Take the specimen and insert the swab or swabs into the upper third of the medium in the small bottle. Cut off the protruding portion of the swab stick with scissors and screw the lid on the bottle tightly. This usually forces the swab down slightly and centers it in the transport medium. Label the bottle and return it with the swabs enclosed to the laboratory as soon as possible. Keep specimens in refrigerator until ready for shipment.

The swabs are sterile. They have been treated with charcoal to improve the conditions for culture.

Laboratory Handling

Grasp the end of the short swab stick firmly with a long-shanked artery forceps and apply swab to culture media in the usual way. It is important that material from the swab itself is applied to the medium surface because adhering transport medium may be deposited on a culture plate and give a false impression of a satisfactory inoculum.

amount in all batches of agar so far examined. This inhibitor becomes of considerable significance because of the absence of protein nutrients in the transport medium.

A transport kit has been described by Stuart (*10*), and full instructions for its preparation and use are given above.

Application of Transport Method

Gonorrhea

Culture is essential in diagnosing gonorrhea only in females; thus consideration is restricted to cervical and urethral swabs from female patients. The two swabs from each patient are placed in the same transport bottle and are cultured together, generally on Peizer's medium prepared approximately as described by Trowbridge and McConkey (*13*) except that Difco supplement B is replaced by a locally prepared yeast extract. Colonies are identified when necessary by the oxidase reaction, and fermentation tests are carried out as a routine.

The relative inefficiency of smear examination is adequately demonstrated in table 1, but there has never been any suggestion from physicians that the combination of smear and culture failed to detect any suspected clinical case.

Table 2. Effect of transport time on culture results [1]

Time, in hours	Number of positives				Percent positive by culture
	Total positive	Culture positive, smear positive	Culture positive, smear negative	Culture negative, smear positive	
Under 24	279	106	163	10	96.4
24–48	480	199	191	90	81.3
48–72	61	21	11	29	52.5
72–96	45	20	16	9	80.0
96 upward	18	6	4	8	55.6

[1] All specimens received September 1953 through June 1955.

Indeed, recent work by Crookes and Stuart (*14*) shows that culture alone, aided by a selective agent to eliminate overgrowth of coliforms, can be depended on to identify practically all infections from patients under adequate clinical control.

The effect of transport time on culture results was assessed in earlier work (table 2), but the percentages have no absolute significance. The decided drop at the 48- to 72-hour period is apparently contradicted by the rise between 72 and 96 hours. The probable explanation of this peculiar finding is that a more interested and cooperative individual took most of the latter specimens. The figures, however, support the belief that culture is still very much worthwhile even after a swab has spent 4 days in transport medium, and these findings have now been considerably improved by a new culture technique (*14*).

Trichomoniasis

Investigation for the presence of *T. vaginalis* is carried out routinely on all specimens received for gonococcal culture and in addition on all vaginal swabs submitted for general or special investigation. After cultures have been prepared, a small amount of material from the swab is suspended in a drop of normal saline on a slide. A coverglass is applied and the preparation is viewed by reduced illumination or by dark field for active trichomonad flagellar movement. In an earlier series of 400 consecutive specimens examined in parallel by a careful staining technique and by the above method, all clinical infestations were detected by the latter. Table 3 shows the findings in our gonococcal culture section over the past 4 years.

Dysentery

The chief value of the transport medium is its applicability to rectal swabs. The value of rectal swabs in the diagnosis of dysentery has been emphasized by Cruickshank and Swyer (*15*), and by Yannet, Leibovitz, and Deutsch (*16*), though their general usefulness has been doubted by Thomas (*17*) and decried by Shaughnessy, Friewer, and Snyder (*18*) for enteric investigations. Most workers agree that the rectal swab to be satisfactory must be kept moist and brought to the laboratory within 6 hours or so. Yet, in a dysentery outbreak in an isolated community the doctor or public health worker, coming perhaps from some considerable distance, may with luck obtain a few specimens of feces but can never hope to investigate at all adequately the incidence or epidemiology of the disease unless he can take rectal swabs. These can readily be collected from a large number of people within a short time, but they can rarely be brought or sent to the laboratory in less than 24 hours.

Experimentally, the transport medium has regularly been found effective in maintaining viability of *Shigella* and *Salmonella* species on rectal swabs for more than 48 hours and has even been found superior to parallel feces specimens up to 4 days. The results of a recent field experience are shown in table 4. Insufficient parallel specimens were received for statistical analysis, but the physicians' increasing

Table 3. Identification of Trichomonas vaginalis from cervical and urethral swab specimens in transport medium

Year	Number of specimens	Number positive	Percent positive
1954	3,039	730	24
1955	3,243	675	21
1956	3,513	739	21
1957	5,042	1,559	31

Table 4. Field experience in Sonne dysentery outbreak [1]

Type of specimens	Number of specimens	Positive specimens	Percent positive
Parallel specimens			
Feces	19	11	58
RST	19	13	68
Individual specimens			
Cases and contacts:			
Feces	143	72	50
RST	99	65	66
Convalescents and carriers:			
Feces	34	2	6
RST	349	36	10

[1] Transport time averaged 2 days.

RST—rectal swab in transport medium.

enthusiasm for the transport rectal swab is revealed in its increased use for convalescents and carriers in the later stages of the outbreak.

During 1956 and 1957, 4,059 specimens were examined in the central provincial laboratory specifically for dysentery organisms. Of 3,469 feces, 100, or 2.9 percent, were positive; of 522 rectal swabs in transport medium, 32, or 6.1 percent, were positive, and of rectal swabs in ordinary tubes, 68 were all negative. (*Shigella sonnei* and *Shigella flexneri* were isolated in approximately equal numbers.) Such evidence suggests that rectal swabs in the transport medium are not inferior to specimens of feces for the isolation of *Shigella* species. Although the method has never been recommended for the investigation of *Salmonella* infections, the following strains were coincidentally isolated from these rectal swabs received in the transport medium; 1 *S. paratyphi B*, 7 *S. typhimurium*, and 1 *S. tennessee*. The effect of time of transport of rectal swabs under field conditions could be assessed in 228 instances when this time was known accurately. For 105 it was one day, and for 123, two or more days. The incidence of detection of *Shigella* was exactly 11 percent in each group.

Haemophilus Infections

Cooper (*11*) has shown experimentally that *Haemophilus influenzae* and *H. pertussis* remain alive in the Stuart swab kit for 28 days, but no figures are available from any large-scale field investigation. Dr. H. Robertson and Dr. H. O. Dillenberg, from the Provincial Public Health Laboratory in Saskatchewan, have provided me with their findings in a small outbreak of whooping cough. From 29 specimens received as swabs, cough plates, or serum slants, *H. pertussis* was isolated only once, whereas from 17 specimens received as swabs in the transport medium *H. pertussis* was isolated in 10 cases. Dillenberg also mentioned that he was able to culture *H. pertussis* from such swabs after holding periods of 3 and 4 days. *Haemophilus vaginalis*, recently described by Gardner and Dukes (*19*), also appears to be a difficult organism to investigate. Amies and Jones (*20*) in this laboratory have succeeded in isolating it from 19 out of 371 cervical swabs submitted in transport medium for routine gonococcal culture.

General Throat and Superficial Infections

The value of the described transport medium for throat pathogens has been more than adequately stressed by Cooper (*11*). In this laboratory over the last 2 or 3 years the transport medium has been recommended on various occasions to city and health unit medical officers. These occasions have generally concerned outbreaks of particular infections, such as diphtheria and beta hemolytic streptococcal disease, particularly when a considerable holding period was inevitable between the taking of the specimens and their delivery to the laboratory. In 1957 a total of 1,020 such specimens were received, and from these 230 *Streptococcus pyogenes*, 344 *Staphylococcus pyogenes*, and 23 *Diplococcus pneumoniae* were reported as probably significant pathogens. Nine of these pneumococci were isolated in pure culture from eye swabs, one after 4 days of transport time. This finding may indicate the value of the transport medium in such specimens from which significant growths are often difficult to obtain.

Whether or not the transport medium is advantageous in ordinary throat infections is debatable. Over the 4-month period January to April 1957, 1,180 throat swabs were received, as ordinary swabs and as swabs in transport

medium, from out-of-town areas at transport distances of 1 to 4 days; 640 swabs were received in transport medium and 540 as ordinary swabs. The area distribution of these specimens was approximately equivalent. *Streptococcus pyogenes* was isolated from 19 percent of the ordinary swabs and from 25 percent of the transport medium swabs. This difference is of doubtful significance, but it might be considered in relation to a parallel observation that the percentage of isolations of streptococci from throat swabs in transport medium remained constant regardless of whether the specimens had been in transit 1, 2, 3, or more days, whereas there appeared to be a considerable drop in the recovery of such organisms from ordinary swabs more than 2 days old.

Because of the enthusiasm of clinical workers for the transport medium, however, it became necessary to minimize these differences in order to protect the laboratory from an inordinate amount of medium making and to restrict the use of transport outfits to health officers in distant areas or under special circumstances. Since the medium and transport outfit are now commercially available, this attitude may have to be revised.

Disadvantages of the Transport Medium

A little extra care must be used by technicians in extracting the swab from the transport jelly, and a trivial delay is imposed during inoculation of culture media by the manipulation of the short swab. Of more significance is the occasional apparent multiplication of gram-negative bacilli on transport swabs even in the absence of recognized nutrients in the transport medium. This is suggested by the finding of heavy growths of coliform bacilli (mainly *Escherichia* and *Aerobacter* species) from about 5 percent of throat swabs in transport media compared with about 1 percent from ordinary swabs. If coliforms in large numbers can interfere with the isolation of hemolytic streptococci they may be even more troublesome with gonococci, especially because they are likely to be more common on swabs from the genitalia. This supposition, raised only after the recent observations on throat swabs, has been supported by a study of laboratory records. Of 100 consecutive culture "failures" over several years, at least 50 were associated with heavy growths of coliform bacilli.

Such observations led to the search for a selective agent which would specifically inhibit coliform bacilli while allowing the growth of all desired pathogens. This agent was particularly difficult to find in connection with *Neisseria*. Search for an agent suitable for incorporation into transport media was soon abandoned. Standardization of the time of exposure is essential to the use of any bacteriostatic agent, and transport time of specimens is completely uncontrollable. In culture plates, however, aerosporin (*A*) (polymyxin B sulfate) has been found to have in low concentrations a regular and specific differential activity between coliforms and pathogenic *Neisseria*, inhibiting the former and leaving the latter unaffected. The beneficial effect on the culture of gonococci achieved by adding sterile aerosporin solution to a final concentration of 1 microgram (10 units) per milliliter in Peizer's medium has been described (*14*), but this technique cannot easily be applied to the isolation of other bacteria. Blood agar plates are generally used for this purpose. The preparation of special batches of this routine medium with aerosporin incorporated would be impracticable in most laboratories because of its irregular use, and parallel cultures on routine and aerosporin media would be expensive and tedious.

Surface impregnation of one-half of a blood agar plate with a few drops of a 25-microgram (250 units) per milliliter of aerosporin solution, prepared by appropriate dilution in water

Table 5. Application of aerosporin technique to selected throat swabs in 89 specimens [1]

Organisms	Routine [2]	Aerosporin [3]
Coliforms	29	[4] 11
Proteus	2	1
Streptococcus pyogenes	4	7
Staphylococcus pyogenes	11	25
Pneumococcus	2	5

[1] Transport time averaged 2 days.
[2] One-half ordinary blood agar plate.
[3] One-half same blood agar plate after surface application of 250 units/ml. of aerosporin.
[4] Distinctly inhibited in 6 specimens.

of the sterile powder provided for intramuscular use, is a simple and effective compromise. This is done prior to inoculation, which is carried out as soon as the plate has dried. Table 5 shows the results from 89 throat swabs, known or suspected to contain coliform bacilli, cultured on such media. Each swab was spread uniformly over the culture plate, one-half of which was the aerosporin test, the other half the routine control.

This technique has not been adopted fully into routine use, chiefly because it is only occasionally necessary. The incidence of coliform bacilli is rarely high enough to cause significant trouble, but whenever necessity has arisen the technique has proved satisfactory. All common respiratory pathogens except *Haemophilus* strains can be effectively isolated by this special technique. *Haemophilus* strains, of course, can still be recovered from the untreated portion of the plate in the usual manner.

Summary

Laboratory experience over a number of years in the use of a non-nutrient transport or holding medium for bacteriological swabs has been described. The method is simple in clinical use, easy in laboratory manipulation, cheap, and of wide applicability. Its greatest value is in the transport of delicate organisms such as *Neisseria*, *Trichomonas*, and probably *Haemophilus pertussis*, and of *Shigella* species on rectal swabs when such specimens are desirable or necessary. It has a further application certainly in the transmission of eye swabs and under special circumstances in the investigation of throat and superficial infections.

The most delicate bacteria appear to survive uniformly over a 24-hour transport period and with considerable regularity up to 48 hours. Even up to 4 days transport time, swab specimens for the diagnosis of gonorrhea in females yield more positives by culture than do corresponding smears by microscopy. Sturdier bacteria survive better.

In general the absence of nutrients from the transport medium insures that the bacterial status quo of specimens is well preserved, but very occasionally coliform bacilli appear able to multiply even in this environment. Whenever this is likely to be a nuisance it can be counteracted easily after the transport swab reaches the laboratory by the use of aerosporin (polymyxin B sulfate) in culture media, either by incorporation or by surface application.

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SUPPLY REFERENCE

(A) Sterile Intramuscular Powder, manufactured by Burroughs, Wellcome and Co.

Study of Back Supports for Premature Infants

The effect on premature infants of a diaper-roll support to the lower back has been the subject of a research study conducted jointly by the Division of Nursing Resources, Public Health Service, and Bellevue Hospital, New York City. Data were collected at Bellevue from April to November 1958.

Round-the-clock observations of the behavior of 30 premature infants receiving routine care and a like number supported by diaper rolls have indicated thus far that the use of the roll does not result in weight gain of the prematures but may contribute to the well-being of the infants by increasing sleeping time and reducing crying. Analysis of the data, now underway, may produce new knowledge on premature infant behavior for nurses, physicians, and social scientists.

The idea for the diaper roll was conceived by Eileen Hasselmeyer, R.N., M.S., nurse consultant with the division, who was chief investigator of the study. She had noticed, several years ago, that premature infants pushed, wriggled, or squirmed until they achieved a position giving support to the lower back.

Miss Hasselmeyer has been awarded a National League for Nursing fellowship for 2 years of study toward a doctorate in the nurse education department of New York University.